

# Inactivation of an ABC transporter, *mcyH*, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806

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**The cyanobacterium *Microcystis aeruginosa* is widely known for its production of the potent hepatotoxin microcystin. Microcystin is synthesized nonribosomally by the thiotemplate function of a large, modular enzyme complex encoded within the 55 kb microcystin synthetase (*mcy*) gene cluster. Also encoded within the *mcy* gene cluster is a putative ATP binding cassette (ABC) transporter, McyH. This study details the bioinformatic and mutational analyses of McyH and offers functional predictions for the hypothetical protein.**

## Hypotheses

It is hypothesized that *mcyH* encodes an ABC-transporter that is responsible for the biosynthesis and/or transport of Microcystin.

## Methods

The *mcyH* gene has been characterized bioinformatically via structural, functional and phylogenetic analyses. In addition, an *mcyH* null mutant has been engineered and characterized with respect to its ability to produce and export microcystin. The McyH enzyme has been heterologously expressed in *E. coli*, purified and used to raise anti-McyH antibodies. These antibodies have been used in immunoblotting experiments to investigate McyH expression in mutant and wild-type strains of *M. aeruginosa*.

## Results

The McyH transporter is putatively comprised of 2 homodimers, each with an N-terminal hydrophobic domain and a C-terminal ATPase. Phylogenetically, McyH was found to cluster with members of the ABC-A<sub>1</sub> subgroup of ABC ATPases, suggesting an export function for the protein. The *mcyH* null mutant strains were unable to produce microcystin. Whilst the *mcyH* deletion had no apparent effect on the transcription of other *mcy* genes, the complete microcystin biosynthesis enzyme complex could not be detected in *mcyH* mutant strains. Expression of McyH was reduced in *mcyA* and *mcyB* mutants and completely absent in the *mcyH* mutant.

## Conclusion

By virtue of its association with the *mcy* gene cluster and the bioinformatic and experimental data presented in this study, we predict McyH functions as a microcystin exporter and is, in addition, intimately associated with the microcystin biosynthesis pathway.